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Biomimetic High Density Lipoprotein Nanoparticles For Nucleic Acid Delivery

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Abstract

We report a gold nanoparticle-templated high density lipoprotein (HDL AuNP) platform for gene therapy which combines lipid-based nucleic acid transfection strategies with HDL biomimicry. For proof-of-concept, HDL AuNPs are shown to adsorb antisense cholesterylated DNA. The conjugates are internalized by human cells, can be tracked within cells using transmission electron microscopy (TEM), and regulate target gene expression. Overall, the ability to directly image the AuNP core within cells, the chemical tailorability of the HDL AuNP platform, and the potential for cell-specific targeting afforded by HDL biomimicry make this platform appealing for nucleic acid delivery.

Keywords

high density lipoprotein; gold nanoparticle; biomimetic; nucleic acid delivery; gene therapy

Nanoparticle (NP) formulations of short nucleic acids (NAs) (e.g. antisense DNA, siRNA, microRNA) have emerged as promising approaches for cellular NA delivery and gene therapy.^{1–6} Some of the most studied strategies rely upon the assembly of NAs with lipids

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Supporting Information Available.

Detailed materials and methods; Western blot data for HIF-1 α ; UV-Vis spectra of HDL AuNPs and chol-DNA-HDL AuNPs; gel electrophoresis results of the nuclease protection assay and conjugate physiologic stability; cytotoxicity data; and supporting RT-qPCR data are presented. This material is available free of charge via the Internet at <http://pubs.acs.org>

and polymers to form liposomes or lipoplexes and polyplexes, respectively.^{2, 3, 7} Lipid-based and other NPs are made multifunctional in attempts to target specific cell types, facilitate cellular uptake, and to mediate the delivery of NA cargo to desired sub-cellular locations, such as the cytoplasm.^{8–10} Often taking direction from nature, many chemical and biological strategies have been employed to overcome each of these hurdles associated with efficient cellular NA delivery.^{11, 12} In addition to lipid- and polymer-based carrier modifications, covalent attachment of lipids, notably cholesterol, directly to NAs has been shown to enhance cellular uptake and target gene regulation even in the absence of carriers.^{13–16} Intriguing recent data demonstrate that intravenously administered cholesterylated nucleic acids (chol-NAs) self-assemble with high density lipoproteins (HDLs), natural phospholipid-rich cholesterol transporters,¹⁷ to deliver adsorbed chol-NAs to cell types targeted by HDL for gene regulation.^{15, 16, 18–22} Overall, this implies that chol-NA-HDL hybrids effectively navigate each of the cellular barriers to NA delivery and that a *de novo* synthesis strategy combining HDL biomimicry with cholesterylated NAs could be used for gene therapy. In this Letter, we report the synthesis of biomimetic HDL nanoparticles based upon a gold nanoparticle template (HDL AuNPs), and investigate their ability to adsorb cholesterylated antisense DNA (chol-DNA). Facilitated by the presence of the AuNP core, we assessed the cellular uptake of chol-DNA-HDL AuNP conjugates. Finally, we utilized a well-characterized and inducible cellular model to investigate the delivery of functional chol-DNA to the cytoplasm of cultured prostate cancer cells and resultant target gene regulation.

Our group²³ and others²⁴ recently synthesized biomimetic spherical HDL nanostructures that closely mimic the size, shape, and surface chemistry of naturally occurring mature spherical HDL. Importantly, these nanoparticle platforms provide a degree of synthetic control over biomimetics of mature spherical HDL that is difficult to achieve using more conventional self-assembly and biological approaches.²⁵ Toward a nanoparticle-based HDL therapeutic, our group utilized gold nanoparticles (AuNPs, 5 nm in diameter) as a scaffold to control the size, shape, and surface chemistry of spherical HDL AuNPs. HDL AuNPs tightly bind the fluorescent cholesterol analogue, 25-[N-[(7-nitro-2-1,3-benzoxadiazol-4-yl)methyl]amino]-27-norcholesterol (NBD-cholesterol) ($K_d = 3.8$ nM).²³ HDL AuNPs have ~3 copies of the HDL-defining apolipoprotein A-I (APOAI) on their surface, and an outer leaflet layer of zwitterionic 1-2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC).²³ Based on the tight binding of NBD-cholesterol by biomimetic HDL AuNPs,²³ the known electrostatic complexation of NAs with phospholipids containing phosphocholine headgroups,²⁶ and data supporting spontaneous assembly and effective cellular delivery of chol-NAs by natural HDL species,¹⁸ we utilized the HDL AuNP platform to adsorb chol-DNA (chol-DNA-HDL AuNPs) and measured the capacity of the conjugates for gene regulation in a model system.

High density lipoproteins are responsible for cholesterol transport and transfer between biological compartments.¹⁷ Blood levels of HDL are significantly inversely correlated with the development of cardiovascular disease.²⁷ A process known as reverse cholesterol transport (RCT) is believed to be at least partially responsible for this phenomenon, and is a process through which high density lipoproteins (HDLs) target cholesterol-laden

macrophages within developing atherosclerotic lesions and remove cholesterol from them.^{28, 29} Sequestered cholesterol is then transported by HDL to hepatocytes for eventual biliary excretion.¹⁷ In addition to RCT, HDLs transport cholesterol to rapidly proliferating cancer cells dependent upon cholesterol delivery in order to maintain membrane biosynthesis and integrity.^{30–34} As such, HDLs avidly target cancer cells which over-express HDL receptors.^{30, 31} The increased need for cholesterol uptake by cancer cells has stimulated interest in using recombinant lipoproteins, especially HDL, engineered for therapeutic delivery.^{30, 32–34} Advanced prostate cancer is uniquely dependent on cholesterol delivery through HDL receptors because of the requirement for cholesterol to support both membrane integrity and the acquired ability to proliferate despite medical or surgical castration by endogenously synthesizing testosterone from cholesterol delivered by HDLs.³⁵ Therefore, prostate cancer provides an ideal model in which to test gene delivery strategies leveraging an HDL biomimetic.

We assessed the ability of chol-DNA-HDL AuNPs to regulate target RNA in cultured PC-3 prostate cancer cells. In addition to choosing an appropriate cell line, we felt it imperative to develop an inducible *in vitro* biological model in order to interrogate the gene regulatory function of chol-DNA-HDL AuNPs. For several reasons, we chose microRNA-210 (miR-210) as our model target. First, miRs, a type of small non-coding RNA, are the major post-transcriptional regulators of gene expression,^{36, 37} making therapeutic modulation of this class of genes a problem of general importance. miRs bind 3'-untranslated regions (UTR) on mRNA targets to induce mRNA degradation or abortive translation.³⁷ Second, miR-210 is the most well-known miR induced by hypoxia^{38–40}, a defining feature of cancer, and has been correlated with worsened outcomes in breast cancer.⁴⁰ Furthermore, miR-210 is induced through a well-characterized mechanism involving the transcription factor hypoxia inducible factor-1 alpha (HIF-1 α).⁴⁰ Under normoxic conditions HIF-1 α is unstable and degraded by the proteasome. Under hypoxia, HIF-1 α is stabilized and able to enter the cell nucleus to bind hypoxia response elements (HRE) in target promoters.⁴¹ In our model system, HIF-1 α stabilization was induced by cellular exposure to cobalt chloride (Figure S1) (CoCl₂, [300 μ M]),⁴¹ which then directly increases miR-210 expression through an upstream HRE. Lastly, the E2F transcription factor 3a (E2F3a) is negatively regulated by miR-210, lending itself to analysis as a readout of miR-210 inhibition in this model system.⁴² For prostate cancer, E2F3a has been shown to modulate cell proliferation.⁴³ In liver cancer HepG2 cells, E2F3a overexpression increased apoptosis and was found to increase the expression levels of several proteins, including the signal transducer and activator of transcription family member STAT1.⁴⁴ Taken together, and to clearly demonstrate proof-of-concept of the function of chol-DNA-HDL AuNPs to regulate an intracellular RNA target, CoCl₂-induced stabilization of HIF-1 α in PC-3 cells provided a well-characterized and inducible molecular system for initial study. A scheme of the cell model is presented in the Supporting Information (Scheme S1).

We fabricated HDL AuNPs as described previously (Scheme 1)²³ and then incubated with chol-DNA antisense to miR-210 (A-210) or scrambled control chol-DNA (100:1, chol-DNA:HDL AuNPs). Following 4-hour incubation, chol-DNA-HDL AuNPs were purified by centrifugation and re-suspension in phosphate buffered saline (1X PBS). Dynamic light

scattering was used to measure the size increase of the nanoparticles at each step of the synthetic process. As expected, the hydrodynamic diameter of AuNPs (7 ± 1 nm) increases upon APOAI addition (11 ± 2 nm), HDL AuNP formation (17 ± 3 nm), and chol-DNA addition (27 ± 3 nm) (Table 1). In addition to particle size, the stability and biological activity of nanoparticles depends upon the charge, or zeta potential, of the formed conjugate.⁴⁵ Data presented in Table 1 for citrate stabilized AuNPs, the HDL AuNP, and chol-DNA-HDL AuNPs demonstrate an overall negative zeta potential, and support the adsorption of negatively charged DNA on the HDL AuNP surface. For HDL AuNPs, a negative zeta potential is consistent with the negative charge of APOAI and naturally occurring HDLs.⁴⁶ UV-Vis spectroscopy confirms the stability of the chol-DNA-HDL AuNP conjugates in 1X PBS. A surface plasmon band centered at ~ 520 nm, consistent with disperse rather than aggregated AuNPs,⁴⁷ demonstrates conjugate stability following surface functionalization (Table 1). Furthermore, for the chol-DNA-HDL AuNPs, a strong absorption band at 260 nm is consistent with DNA on the conjugate surface (Figure S2). The number of chol-DNA strands on the surface of chol-DNA-HDL AuNPs was quantified using fluorescently labeled chol-DNA and found to be ~ 13 per HDL AuNP (Table 1).

Fluorophore-labeled APOAI was used to fabricate HDL AuNPs and chol-DNA-HDL AuNPs in order to quantify the number of APOAI molecules bound to the conjugate surface. Data demonstrate that there are ~ 2 copies of APOAI on the surface of the chol-DNA-HDL AuNPs and that APOAI remains bound to the particle surface in the presence of chol-DNA (Table 1). For phospholipids, previous data indicates that an HDL AuNP harbors ~ 80 DPPC molecules.²³ For this study, we synthesized chol-DNA-HDL AuNPs using radiolabeled ^3H -DPPC (10 mol% to unlabeled DPPC) to measure the number of labeled phospholipids in HDL AuNPs and chol-DNA-HDL AuNPs, as well as to measure the presence of excess lipid remaining in solution following final conjugate purification. Each HDL AuNP has ~ 8 ^3H -DPPC molecules. Chol-DNA-HDL AuNPs harbor ~ 3 ^3H -DPPCs, consistent with a loss of DPPC upon chol-DNA addition to the HDL AuNP surface. Finally, measurement of the ^3H -DPPC content of the supernatant after chol-DNA-HDL AuNP purification revealed an absence of residual ^3H -DPPC making it unlikely that cellular delivery of chol-DNA (*vide infra*) is due to residual DPPC. Transmission electron microscopy (TEM) imaging demonstrates the presence of a corona surrounding chol-DNA-HDL AuNPs but not around colloidal AuNPs providing visual evidence of AuNP surface functionalization (Scheme 1).

Encapsulation serves to protect nucleic acids from nuclease degradation in lipoplexes and polyplexes. HDL AuNPs assemble chol-DNA molecules on their surface, raising the possibility that adsorbed chol-DNA is susceptible to nuclease degradation. Accordingly, we assessed the stability of HDL AuNP-bound chol-DNA versus free chol-DNA through a nuclease protection assay (NPA). Solutions of chol-DNA-HDL AuNPs and free chol-DNA were exposed to DNase-I for 15, 30, and 60 minutes followed by gel electrophoresis to isolate and visualize chol-DNA. Data demonstrates that HDL AuNPs protect chol-DNA from nuclease degradation as compared to the free chol-DNA (Figure S3). The enhanced stability of cholesterylated nucleic acids when bound to HDL is consistent with previous work with natural HDL upon *in situ* assembly with cholesterylated siRNA.¹⁸

In addition to stability to nuclease degradation, chol-DNA-HDL AuNPs must remain intact under relevant physiologic conditions. Most importantly, as the surface components of natural HDL species¹⁷ and synthetic HDLs⁴⁸ can exchange with other lipoproteins and serum components, we used gel electrophoresis to test for the presence of chol-DNA on conjugates isolated by centrifugation after incubation in PBS and cell culture media supplemented with 10% fetal bovine serum (FBS) for 24, 48, and 72 hours. In addition, UV-Vis spectroscopy was performed on the conjugates after incubation and isolation to assess particle dispersion. Gel electrophoresis reveals that chol-DNA remains on the surface of the HDL AuNPs after incubation in PBS and cell culture media at all time points (Figure S4). In all cases, UV-Vis spectroscopy revealed an absorption band at ~525 nm which is consistent with dispersed AuNP conjugates (Figure S4). These data provide evidence that the chol-DNA-HDL AuNPs are stable to aggregation and retain chol-DNA in the physiologic matrices required for cellular nucleic acid delivery.

Prior to functional biological studies of the chol-DNA-HDL AuNPs, we evaluated the cellular uptake, sub-cellular localization, and cytotoxic effects of chol-DNA-HDL AuNPs in our cellular model of prostate cancer. Confocal microscopy using chol-DNA-HDL AuNPs synthesized with fluorophore-tagged chol-DNA, revealed that the NPs associated with and rapidly entered PC-3 cells (Figure 1, A–D). At ~4 hours, fluorescence localized predominantly to the cellular membrane. Subsequently, signal re-distributed from punctuate intracellular vesicles and was more homogeneously distributed (8 and 12 hrs). After ~24 hours, fluorescent signal was again concentrated in vesicles. These data suggest that chol-DNA reaches the cytosol following chol-DNA-HDL AuNP delivery. At ~24 hours, appearance of fluorescence within vesicles suggests that the chol-DNA is beginning to be degraded. This time course of events is consistent with theoretical and experimental evidence of fluorescent antisense oligonucleotide uptake in cells.⁴⁹ The chol-DNA-HDL AuNP conjugates provide the opportunity to visualize the sub-cellular localization of the AuNP portion of the conjugate following cell treatment. We performed transmission electron microscopy (TEM) of PC-3 cells 16 hrs after treatment with chol-DNA-HDL AuNPs. Indeed, AuNPs were easily visualized throughout the cell confirming uptake of the AuNP component of the conjugates (Figure 1, E–H). Interestingly, the AuNPs appear to be free of sequestration within endosomal vesicles; however the AuNPs appear to be in discrete collections within the cell at this time point. Finally, the toxicity of the chol-DNA-HDL AuNPs was investigated using a lactate dehydrogenase (LDH) release assay. Following 48 hour treatment, we observed no toxicity above background levels, even at chol-DNA-HDL AuNP concentrations well above that needed for target RNA regulation (Figure S5).

In order to confirm the biological function of the chol-DNA-HDL AuNPs to regulate miR-210 after CoCl₂ induced stabilization of HIF-1 α , we measured miR-210 levels in PC-3 cells treated with A-210 and scrambled chol-DNA-HDL AuNPs by real-time PCR (RT-qPCR). Cell treatments were conducted in serum-free and serum-containing media in order to assess any confounding of the data due to lipoproteins present in serum. As shown in Figure S6, similar results were obtained in serum-free and serum-containing media. A-210 chol-DNA-HDL AuNPs caused an 80% reduction in cellular miR-210 levels compared to HDL-AuNP and scrambled chol-DNA-HDL AuNP controls (Figure 2A). Free A-210 chol-DNA added to cells at approximately equimolar concentration caused a more modest 55%

reduction of the miR-210 levels while free scrambled chol-DNA did not cause an appreciable change (Figure 2A). At 48 hours, miR-210 levels remained low and began to recover by 72 hours after treatment initiation (Figure S7).

To verify the consequences of the miR-210 blockade by A-210 chol-DNA-HDL AuNPs at the target protein level, we performed Western blot analysis of the expression levels of the known miR-210 target, E2F3a.²² RT-qPCR data revealed that cell treatments of 10 and 50 nM chol-DNA-HDL AuNPs (100 and 500 nM free chol-DNA) were not appreciably different (Figure S7); thus, the 10 nM chol-DNA-HDL AuNP (100 nM free chol-DNA) dose was used for experiments to assess target protein regulation. Consistent with previous findings, PC-3 cells expressed E2F3a, which decreased in the presence of CoCl₂ stabilization of HIF-1 α (Figure 2B).⁴² Treatment with A-210 chol-DNA-HDL AuNPs results in a de-repression of E2F3a expression which is superior to that of free cholesterylated A-210 (Figure 2B). Neither HDL AuNPs, nor scrambled chol-DNA-HDL AuNPs, nor free scrambled chol-DNA affected E2F3a levels. Furthermore, our model system provides a means to further delineate the regulation of targets downstream of E2F3a in order to further validate miR-210 regulation through A-210 chol-DNA-HDL AuNPs. As stated previously, STAT1 is reported to be positively regulated by E2F3a.⁴⁴ Accordingly, knockdown of miR-210 and de-repression of E2F3a was hypothesized to result in an increase in STAT1 expression. Indeed, data demonstrates that CoCl₂ treatment and miR-210 up-regulation reduces STAT1 expression, and that de-repression of E2F3a by A-210-chol-DNA-HDL AuNP treatment increases STAT1 (Figure 2B). Overall, the level of regulation of E2F3a and STAT1 through treatment with A-210 is consistent with concept that miR-210 is acting as a rheostat to fine tune gene expression.⁵⁰

Our data provide compelling evidence of the efficacy of hybrid chol-DNA-HDL AuNPs as conjugate agents for controlling gene expression. The conjugates stabilize chol-DNA to nuclease degradation and the conjugates are stable in relevant physiologic matrices. Utilizing an inducible molecular model of HIF-1 α stabilization, chol-DNA-HDL AuNPs enter PC-3 cells, do not demonstrate non-specific cellular toxicity, and function to regulate intracellular miR-210. At the protein level, reduction of intracellular miR-210 leads to the de-repression of E2F3a and STAT1. The HDL AuNP platform is a general one which can be tailored to optimize chemical properties which may enhance the efficiency of gene regulation. For instance, the sequence and type of nucleic acid may be changed to engage potentially more potent mechanisms of gene regulation (e.g. siRNA, miR); the lipid content of the HDL AuNP can be manipulated to enhance nucleic acid binding and release from the surface of the conjugate; and, the final particle size can be tailored to optimize target cell membrane-nanoparticle interactions for NA delivery. In addition, and as demonstrated by others, the core nanoparticle may also be changed in order to develop agents for imaging purposes.^{51–53} Our data indicate that the chol-DNA component of the conjugate enters the cell, disperses, and is then found localized within cellular vesicles. TEM data demonstrate the AuNP component of the conjugates within the cell cytoplasm. Currently, experiments directed at dissecting the intracellular fate of each of the conjugate components and correlating this with gene regulatory function are underway. Interestingly, Skajaa *et al* utilize HDL biomimetic HDLs synthesized upon a quantum dot scaffold (QD-HDL) and

utilize fluorescence resonance energy transfer (FRET) to study dynamic lipoprotein interactions.⁴⁸ Their work demonstrates that the surface chemical components of QD-HDL and native lipoproteins interchange and that the novel properties of the QD core can be leveraged to elucidate the cellular fate of adsorbed biomimetic HDL nanoparticle surface components. Data reported herein demonstrate that over time the chol-DNA-HDL AuNPs are stable in physiologic matrices; however, it is likely that a dynamic equilibrium exists between the surface components of chol-DNA-HDL AuNPs and the components of physiologic matrices and target cells. Differences in the fabrication of the conjugates reported in this Letter and those fabricated by Skajaa *et al*, perhaps most notably the use of the disulfide containing phospholipid reported here, highlight the opportunity for directed tailoring of the HDL AuNP with studies designed, for instance, to ascertain how the AuNP ligand binding energies affect lipid exchange. Finally, taking *in situ* assembly of cholesterylated nucleic acids with HDL as a model,¹⁸ the biomimetic HDL AuNP platform may provide advantages over other non-viral *in vivo* NA delivery strategies with regard to systemic pharmacokinetics, cell targeting through HDL receptor-mediated conjugate uptake, and optimal cellular function. With regard to targeting therapeutic nucleic acids to cells *in vivo*, Wolfrum et al demonstrated that the *in situ* assembly of cholesterylated siRNAs with HDL results in chol-siRNA delivery to cells involved in cholesterol metabolism that express the HDL scavenger receptor B-1 (SR-B1).¹⁸ Due to the expression of SR-B1 by both cancer cells, including prostate cancer,^{30, 35} and cell types involved in reverse cholesterol transport (e.g. macrophages and hepatocytes), molecular targeting through appropriate choice of therapeutic nucleic acids will be imperative. In conclusion, the HDL AuNP platform, a hybrid combining biomimicry with lipid-based NA delivery strategies, mediates efficient cellular nucleic acid delivery and may find utility for the targeted *in vivo* delivery of NA therapeutics for any number of disease processes, including atherosclerosis, inflammation, and cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Fluorescent confocal microscopy and transmission electron microscopy (TEM) for cellular distribution of chol-DNA-HDL AuNPs in PC-3 cells

For both confocal and TEM experiments, HIF-1 α stabilization was induced in PC-3 cells with 300 μ M cobalt chloride (CoCl₂) for 12 hours prior to chol-DNA-HDL AuNP treatment (50 nM conjugate, final). **Left.** Chol-DNA-HDL AuNPs fabricated with fluor-labeled DNA (green) were incubated with cells and imaged at various time points. Keratin (*red*) and nuclei (*blue*) were stained after cellular fixation. Images were taken after 4 (A), 8 (B), 12 (C), and 24 (D) hour incubations with chol-DNA-HDL AuNPs. TEM images were obtained after a 16 hour chol-DNA-HDL AuNP transfection. Arrows indicate AuNPs in the PC-3 cell. Magnifications: (E) 890 X, (F) 2,900 X, (G) 23,000 X, (H) 98,000 X.

Figure 2. RT-qPCR and Western blot assessment of chol-DNA-HDL AuNP-mediated knockdown of miR-210

Note: The dose of the conjugate (i.e. HDL AuNP or chol-DNA-HDL AuNP) immediately follows “HDL” and the dose of free or HDL AuNP-adsorbed chol-DNA immediately follows “DNA”, as appropriate, for each treatment. (A) At the miR-210 level, A-210 chol-DNA-HDL AuNP treatment significantly reduces miR-210 expression in the setting of CoCl₂, both in comparison to HDL AuNP alone, and in comparison to an approximately equimolar dose of the free A-210 chol-DNA ($P < 0.01$, $n=3$). Data are presented as mean $\pm$ SE. (B) Western blot of E2F3a, a target of miR-210, demonstrates that A-210 chol-DNA-HDL AuNP treatment de-represses E2F3a at 10 nM dose (~ 100 nM chol-DNA) (top). STAT1 expression is regulated by E2F3a and Western blot demonstrates that de-repression

of E2F3a results in increased STAT1 expression (middle). Beta actin was used as protein loading control (bottom).

Scheme 1. Synthesis of chol-DNA-HDL AuNPs and characterization

1) An aqueous solution of colloidal gold nanoparticles (AuNPs, 5 ± 0.75 nm diameter) is mixed with apolipoprotein A-I (APOAI). 2) A mixture of phospholipids is then added to the surface of the AuNPs to form biomimetic HDL AuNPs. The HDL AuNPs are purified by centrifugation and re-suspension in water. 3) chol-DNA-HDL AuNPs are synthesized by adding chol-DNA to purified HDL AuNPs. After incubation, the chol-DNA-HDL AuNP conjugates are purified by centrifugation and re-suspended in phosphate buffered saline. Transmission electron micrographs (TEM) of an individual 5 nm AuNP and chol-DNA-HDL AuNP are shown.

Table 1
Characterization data for each step of the synthetic process to chol-DNA-HDL AuNPs

The hydrodynamic diameter of chol-DNA-HDL AuNPs gradually increases as surface components are added. The UV-vis λ_{max} remains at 520–525 nm throughout the synthetic process demonstrating stability and dispersity of the formed conjugates. Zeta potential measurements demonstrate the negative charge of the HDL AuNPs, which increases upon chol-DNA adsorption. By labeling APOAI with a molecular fluorophore, the molar ratio of APOAI:AuNP was determined at each step of the synthetic process where APOAI is present. Similarly, the molar ratio of chol-DNA was determined for the final chol-DNA-HDL AuNPs using DNA modified with a molecular fluorophore. Hydrodynamic diameters are shown as diameter $\pm$ SD.

	AuNP	APOAI-AuNP	HDL AuNP	Chol-DNA-HDL AuNP
Size (nm)	7 $\pm$ 1	12 $\pm$ 1	16 $\pm$ 3	27 $\pm$ 4
UV-Vis λ_{max} (nm)	519	518	525	524
Zeta Potential (mV)	−13 $\pm$ 0	N/A	−31 $\pm$ 2	−36 $\pm$ 2
APOAI:AuNP Molar ratio	N/A	3 $\pm$ 0	2 $\pm$ 0	2 $\pm$ 1
Chol-DNA:AuNP Molar ratio	N/A	N/A	N/A	13 $\pm$ 1